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14. ABSTRACT
Accurate diagnosis of traumatic brain injury (TBI) and prediction of outcome following treatment is a key factor in head-trauma management. One of the serious consequences of TBI is diffuse axonal injury (DAI). Magnetic Resonance Imaging (MRI) based Diffusion Tensor Imaging (DTI) is an ideal modality to detect DAI as DTI can detect and quantify small alterations in local diffusion patterns due to axonal injury. Preliminary analysis of DTI data acquired from TBI patients and 10 normal control subjects suggests that (1) it is possible to detect, localize and quantify injured regions with high statistical significance by comparing the voxellated diffusion anisotropy maps of individual TBI patients to those of the control group after all data have been normalized, and (2) it is possible to identify brain pathways disrupted by injury and to quantify disruptions in individual subjects by DTI tractography. This quantification provides a metric to monitor the longitudinal progression of TBI in response to treatment.

15. SUBJECT TERMS
Traumatic Brain Injury, Diffuse Axonal Injury, Diffusion Tensor Imaging, Tractography, DTI Quantification

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PROGRESS REPORT

INTRODUCTION

Accurate diagnosis of traumatic brain injury (TBI) and prediction of outcome following treatment is a key factor in head-trauma management. One of the serious consequences of TBI is diffuse axonal injury (DAI) or white matter injury induced by sudden acceleration/deceleration and/or rotational/vibrational forces that cause a shearing of nerve fibers [Strich 1961, Adams et al. 1982, Arfanakis et al. 2002, Huisman et al. 2004]. White matter integrity is critical to brain function. Head injury sustained near an explosion commonly induces significant DAI, which has been identified as one of the key reasons for permanent disability or death and in general can be very debilitating, leading to a wide range of neurological impairments from mild memory deficits to a persistent vegetative state. Though computed tomography (CT) and Magnetic Resonance Imaging (MRI) are routinely employed to evaluate trauma and DAI, several reports suggest that the existing imaging protocols are unable to detect the full extent of injury and likely to underestimate the consequences of DAI, resulting in a poor correlation between diagnosis and final outcome [Gentry et al. 1988, Kelly et al. 1988]. Diffusion Tensor Imaging (DTI) is a recently developed imaging modality that is very sensitive to detect axonal disruptions in terms of local changes in the diffusion pattern of water molecules within and around bundles of axons. The degree of diffusion anisotropy, called Fractional Anisotropy (FA), is a sensitive parameter to characterize local diffusion. It has been hypothesized that the consequences of TBI on DTI would be a decrease in FA resulting mainly from a decrease in diffusivity along the principal direction. Thus FA changes would be a marker of injury. In addition to FA changes, a thorough evaluation of DAI requires knowledge of specific brain connections that may be disrupted by the injury. The scope of this research project was to use DTI to first locate and quantify injured regions in TBI patients via changes in the FA compared to a normal group, and then use normalized tractography to quantify disruption along specific brain pathways likely to be affected by the injury. A key objective was to quantify all tractography in the 3D head-space of individual TBI subjects to enable progressive, objective monitoring of individuals.

BODY

Significant progress has been made along all items of the work statement as described below. However we would like to point out that in our original proposal, we had requested a second year optional funding to perform an additional key task (task 8 in the original proposal), to conduct follow-up studies in all subjects and correlate DTI findings to neuropsychological tests representing the outcome of any TBI therapy. This task for the second optional year was not funded.

SOW items and Progress

1. *<Identify 12 head-trauma subjects and 12 control subjects with existing MRI-DTI data. Transfer data to Dr. Singh's laboratory>*

Twelve appropriate trauma subject for whom DTI data had been acquired at the LAC-USC County Hospital as part of their routine medical care were identified by Drs. Gruen and Zee and their DTI data was transferred to Dr. Singh's laboratory. Ten normal subjects with existing DTI data who could serve as a control group for the trauma subjects were also identified in Dr. Singh's database.

2. *<Create DTI database from above with all identifiers removed. Include clinical scores in database>*:

DTI data from the 12 trauma subjects and 10 normal controls has been input in the DTI database in Dr. Singh's laboratory.

3. *<Compute Fractional Anisotropy (FA) images and whole-brain tractography including fiber-crossing corrections as needed>*

The FA images have been created for all 22 subjects (12 trauma, 10 normals) using a one-fiber per voxel streamline tractography approach. Although we have done much work to implement ICA (Independent Component Analysis) based multi-fiber per voxel algorithm in streamline tractography (Singh et al. 2006, Singh et al. 2009a), normalizing these multi-fiber tractographies is still a challenge and we continue to pursue this through other funding mechanisms.

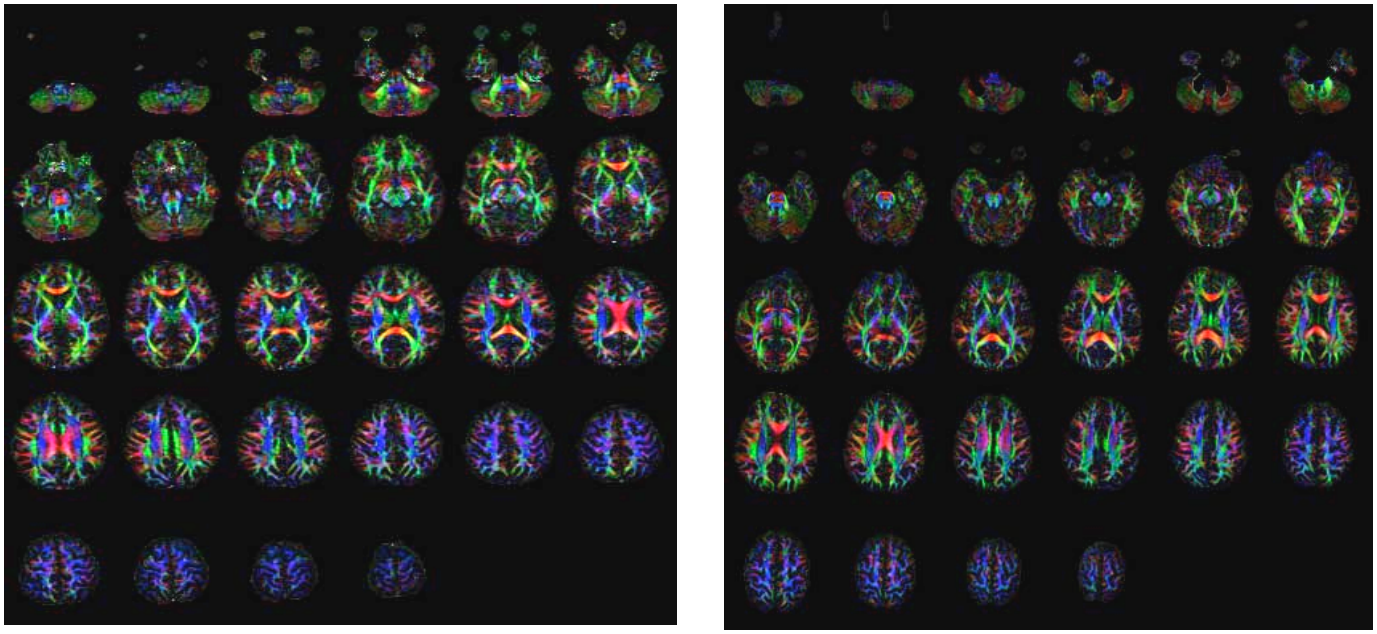

Fig 1: FA map of a 28-slice brain volume. (left) normal control, (right) TBI patient. Note a FA reduction in the genu of the TBI patient.

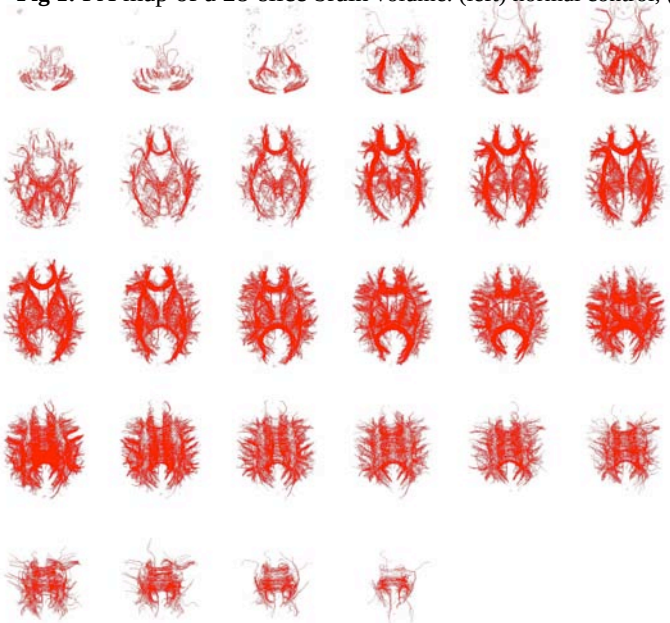

Fig. 2: An example of whole-brain tractography. All tracts intersecting a slice are shown for 28 slices in a normal subject.

As part of their routine clinical evaluation, DTI data were acquired from certain trauma subjects identified by Drs. Gruen and Zee on a 1.5T MRI (GE EXCITE) system using a whole-brain single shot Echo Planar Imaging (EPI) sequence with repetition time TR=10.3s, field-of-view 26cm, 128x128 matrix, 28 contiguous4mm thick slices, 25 isotropic gradient directions at b=1000s/mm^2, one b=0 image, and number of excitations (NEX)=2 for a total acquisition time of 7min 50s. In-house software was then used to estimate the tensor, various diffusion parameters and whole-brain tractography. An example of the FA map for a normal subject is presented in **Fig. 1(left)**. Color-coding is used to demarcate the three main directions Anterior-Posterior (green), Superior-Inferior (blue), Left-Right (red). Using the RGB color scale, non-orthogonal directions are shown as a combination of red, green, and blue. An example of the FA map for a trauma subject is shown in **Fig. 1(right).** From ancillary clinical information, it was known that this subject suffered injury to the left frontal brain and a reduction in the FA in the genu can be visualized in **Fig. 1(right)** (arrow). However, it is not possible to quantify changes, locate all injured regions or determine the actual pathways affected by injury through visual examinations alone.

Whole-brain tractography provides better visualization of connections among regions than the FA maps. An example of 28-slice whole-brain tractography is presented in **Fig. 2** where tracts intersecting individual slices for a normal subject are shown. These images show brain connectivity and can be viewed as a movie to visualize brain connections in 3D. For example, one view of the rendered brain containing the genu is shown in **Fig. 3(left)** and similar rendered views of the TBI subject containing the genu are shown in **Figs. 3(middle) and 3(right)**. Again the disconnections along the genu in this subject can be seen in **Fig. 3(middle)**. However, it is still not possible to quantify connectivity among specified regions from such images.

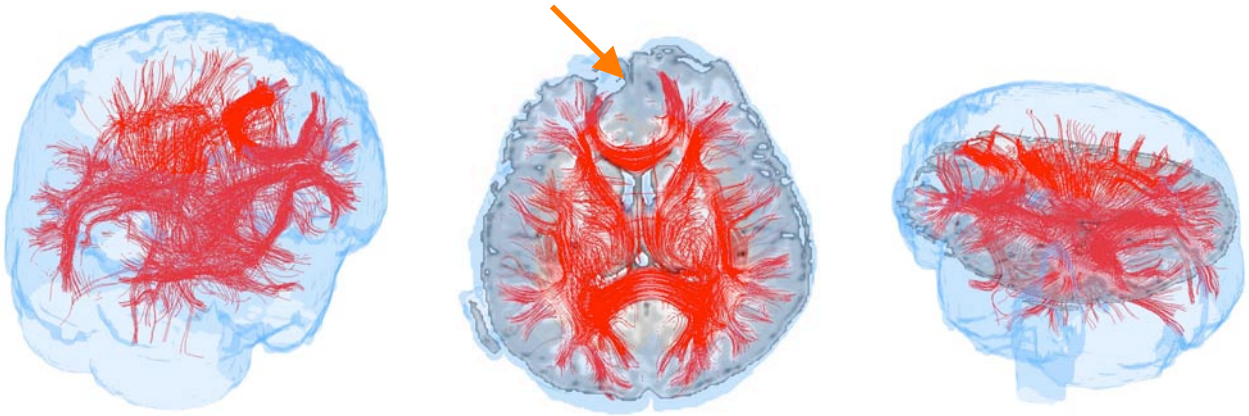

Fig. 3(left): An example of tracts intersecting a specified plane in 3D rendered brain for a normal subject. (middle and right) Same for a TBI subject. The disruption along the genu is now visualized better (arrow). Please note that these images are shown in Neurology convention (right hemisphere displayed at right, left hemisphere at left) whereas Fig. 1 is shown in Radiology convention (right hemisphere at left, left hemisphere at right).

4. *<Identify ROIs within trauma patients and compare ROI connectivity among specified ROIs to same ROIs in control subjects using inverse normalization to define ROIs objectively>*

A customized FA template was created by normalizing individual FA maps of the 10 NC subjects through a two-step procedure relying on Statistical Parameter Mapping or SPM based normalization (http://www.fil.ion.ucl.ac.uk/spm/) of segmented white matter voxels (co-registered to the b=0 images) to the Montreal Neurological Institute or MNI template using a 12 parameter affine/non-linear transformation, followed by refinement in a second step through whole-brain Echo Planar Imaging (EPI) to EPI normalization. FA-template based normalization was then used to map the center points of all voxels in MNI space to each subject's native space by inverse normalization. These inverse mapped coordinates were used as seeds for whole-brain tractography in individual subjects. This procedure ensured that not only were the number of seeds equal in all subjects but also that seeds were distributed at anatomically equivalent locations in the native space of each subject. All tracts from each control subject (streamline tractography, 0.2mm step size, FA>0.2, deflection<45°) were then individually transferred back to the MNI space using forward mapping of every 0.2mm spaced point on each tract. Thus the number of tracts remains unchanged from each subject's native space to standard space. Also this procedure maintains the continuity of individual tracts, does not introduce any additional smoothing, and normalizes for different head sizes, shapes and individual white-matter variations by distributing an equal number of seeds at anatomical equivalent locations in each subject. Then all of these tracts from normalized space were mapped onto the head of individual TBI subjects using a second transformation that relates the MNI space to the individual TBI subject's space. Details of this procedure are described in the attached abstract (Singh et al. 2009b) and full manuscript (accepted with contingencies) in the Journal "*Magnetic Resonance Imaging*" (Singh et al. 2009c). The connectivity between or among specified ROIs for an individual can be quantified by counting tracts between targeted regions (Singh et al. 2007). If injured regions

contain more voxels whose FA is below the threshold than normals (which is likely in TBI), the number of tracts through the injured regions will also be less than normals. Thus by using the injured regions as ROIs to sort tracts, one can identify specific brain pathways along which connectivity is disrupted by the injury and quantify the loss of connectivity by counting and comparing tracts between individual TBI subjects and normals at a specified FA threshold. Examples of reduction in tract counts through regions identified by using FA changes as a signature of injury are presented together with FA comparison results in item 5 below of the statement of work.

5. *<Compare FA values of trauma patients to control group averages within targeted ROIs>*

A T-score based voxel based analysis was conducted to detect statistical significant changes in the FA values of individual TBI subjects and the group averaged FA values of the control subjects (Singh et al. 2009b). The results of this comparison for a TBI subject are presented in **Fig. 4.** The colored spots show regions corresponding to FA decrease in this TBI subject at $t \geq 3.0$, superposed on the subject's FLAIR (**Fl**uid **a**ttenuated **i**nversion **r**ecovery) images. No significant spots were detected where the FA would have increased for the TBI subject.

The FLAIR images mostly reflect edema and it is noteworthy that in most regions, there is partial overlap between the FA changes and FLAIR spots, suggesting that the FA changes occur only over a portion of the FLAIR regions. However there were also regions where there was no overlap between the two modalities. Thus FA changes, which directly reflect changes in axonal integrity and configuration, provide information that could not be achieved through FLAIR imaging alone.

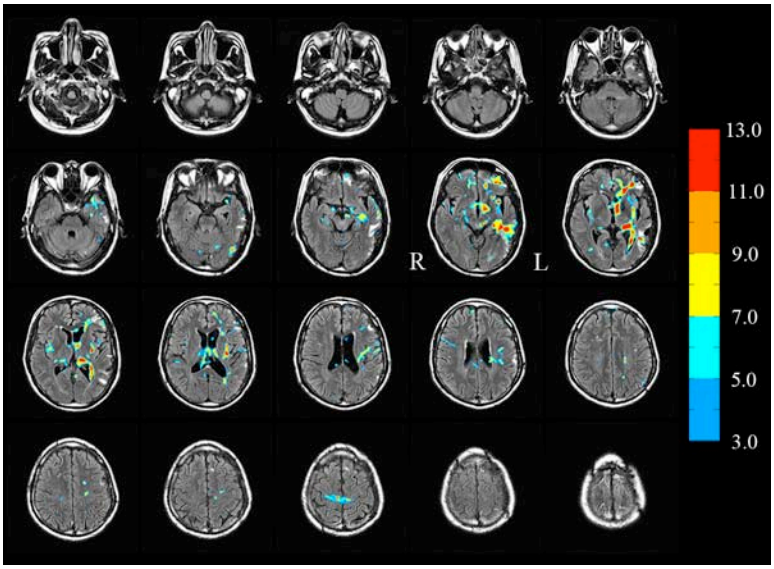

Fig 4: Example of FA reduced regions corresponding to $t \geq 3.0$ (see color bar) superimposed on the FLAIR images of TBI subject 1. An extent threshold $k \geq 12$ was also used to identify clusters. Some FA-reduced regions overlap completely, others partially and some do not overlap with the FLAIR spots. (In this montage, the left hemisphere L appears at the right in each image).

Specific brain pathways compromised by the injured regions can now be sorted from the whole-brain normalized tracts and quantified by using the FA-changed regions as ROIs. An example of the specific pathways affected by one of the prominently injured regions showing injury to the fornix, fronto-occipital and inferior longitudinal fascilus, is presented in **Figs. 5** where all tracts from the controls were mapped onto the TBI subject's head. An example of tracts from a control subject is also shown in **Fig. 5**.

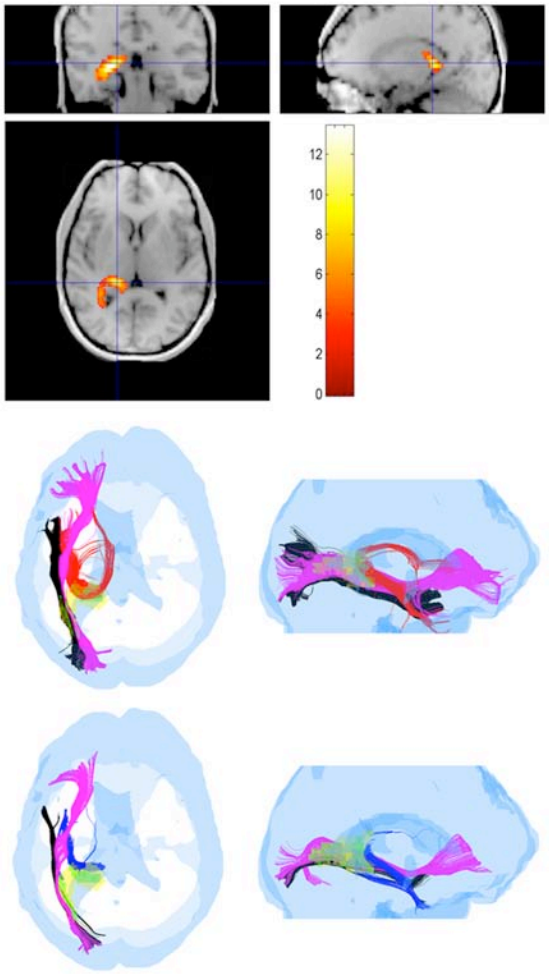

Fig. 5: Three pathways, hippocampal/fornix (HC/FX), inferior fronto-occipital (IFO) and inferior longitudinal fasciculus (ILF) identified as crossing the voxels of the highest *t*-score ROI in a TBI subject (subject 1). (top panel) Coronal, sagittal and axial views of the ROI with color-coding of the *t*-score as indicated in the color bar. (middle row) The three pathways (HC/FX-red, IFO-magenta, ILF-black) in a normal subject shown in axial and sagittal views. (bottom row) The same three pathways (HC/FX-blue, IFO-magenta, ILF-black) in a TBI subject.

6. *<Correlate connectivity and FA differences between individual and control group to clinical outcome>*

Only a qualtitative correlation could be done as the GCS scores of the TBI subjects used in this study were all clustered around 13 and thus not suitable for a correlation analysis. Quantitative neuropsychological testing was not funded. Qualitatively it was observed that most of the TBI subjects complained about short-term memory problems and in all of those subjects, a significant reduction was seen in their fornix connectivity.

KEY RESEARCH ACCOMPLISHMENTS

- DTI data that are uniquely capable of identifying brain regions where traumatic brain injury has occurred were acquired from 12 TBI subjects and 10 normal control subjects.
- A novel method has been developed to normalize all DTI data in one 3D space to identify and quantify the injured regions in individual TBI patients by voxel based comparison of an individual's Diffusion Fractional Anisotopy (FA) map to a normal control group.
- Novel methods have been developed to normalize whole-brain tractography to identify key pathways in

the brain affected by injured regions in individual TBI patients compared to a group of normal subjects, and to quantify the effect of injury on specific brain connectivity by using a metric based on normalized tract count.

- Results suggest statistically significant reduction in FA and brain connectivity along specific pathways in individual trauma subjects compared to a normal control group.

REPORTABLE OUTCOMES

Presentations and Publications of Proceedings:

1. Singh M and Jeong J-W, "Localization and quantification of injured regions and affected pathways in the 3D head-space of individual TBI subjects using DTI tractography with automatically generated ROIs" *Proceedings, 17-th Scientific Meeting, Int. Soc. Magnetic Resonance In Medicine,* Hawaii, April 2009. **(Appendix 1)**

2. Singh M, Jeong JW, Hwang D, Sungkarat W, Gruen P, "Novel DTI Methodology to Detect and Quantify Injured Regions and Affected Brain Pathways in Traumatic Brain Injury", *Magnetic Resonance Imaging*, (Accepted with contingencies, 2009).

3. Singh M and Jeong J-W, "DTI-Tractography to Detect and Quantify Brain Pathways Affected in Traumatic Brain Injury" *Proceedings, 16-th Scientific Meeting, Int. Soc. Magnetic Resonance In Medicine*, Toronto, Canada, #2269, May 2008. **(Appendix 2)**

4. Singh M, Invited Speaker, "Detection of White-Matter Pathways affected in TBI by DTI-Tractography", *International Annual Symposium of the Brain Mapping and Intraoperative Surgical Planning Society,* Washington D.C., November, 2007. Published in the conference proceedings. **(Appendix 3)**

5. Singh M, Invited Speaker, "Quantitative DTI with Applications to Traumatic Brain Injury and Alzheimer Disease", *5-th International Annual Symposium of the Brain Mapping and Intraoperative Surgical Planning Society*, Los Angeles, August 2008. Published in the conference proceedings.

Proposals submitted based on this work:

1. Investigator Initiated Research submission (PI: Singh) Period: 7/01/08 to 06/30/2012
   DOD $110,260
   Efficacy of Nicotinamide for the Treatment of Mild Traumatic Brain Injury- Clinical Correlations with DTI-Tractography and Genotype
   Project Goals: To develop DTI tractography for monitoring the efficacy of Nicotinamide treatment in mild TBI. (not Funded)

2. Investigator Initiated Research submission (PI: Singh) Period: 7/01/08 to 06/30/2012
   DOD $137,062
   DTI Tractography and Cognitive-Behavioral Measures to Monitor TBI Progression
   Project Goals: Acquire and process longitudinal DTI data from groups of TBI and normal subjects, detect and quantify brain pathways affected by TBI, correlate to cognitive and behavioral measures and monitor progression over three years. (not Funded)

PERSONNEL INVOLVED

1. Manbir Singh PH.D. PI 2. Peter Gruen M.D., Co-PI 3. Chi-Shing Zee Ph.D. Co-I

4. Edward Grant M.D., Co-I, 5. Jeongwon Jeong Ph.D. Research Associate

CONCLUSION

The main objective of this work was to conduct a feasibility study to determine if Diffusion Tensor Imaging (DTI) is a viable approach not only to detect and quantify the location and extent of Traumatic Brain Injury (TBI) but also to identify pathways in the human brain that might be affected by the injury and to quantify connectivity along these pathways. DTI data were obtained and analyzed from 12 patients who had suffered Traumatic Brain Injury in motor vehicular accidents and compared to DTI data acquired from a group of 10 normal control subjects. Novel methods incorporating unique normalization techniques were developed to compare diffusion based Fractional Anisotropy (FA) maps of individual trauma subjects to a template made from the 10 normal control subjects. These results vividly demonstrate the location and extent of injury in individual subjects, where the degree of injury is quantified by a statistically significant FA change metric. Unique methodology was developed to normalize whole-brain tractography of all subjects to a common space and then map tracts onto the 3D space of any individual subject such that an individual TBI subject's tracts can be compared to normals and differences quantified in the subject's own head-space. This procedure allows us to demarcate brain pathways in individual trauma patients affected by injury and to quantify the degree of loss in connectivity along specific pathways **The results identify and quantify connectivity losses along specific pathways in each of the 12 trauma subjects studied and point to specific cognitive deficits that may arise from each injured regions.**

The results demonstrate, perhaps for the first time, the feasibility of using DTI to not only detect the location and extent of traumatic injury but also to **quantify the consequences of each injured region to specific brain function in an individual TBI subject**. As axonal connectivity is integral to brain function, it is likely that this work will demonstrate a direct link between injury and specific cognitive function that could be quantified by neuropsychological examinations. Ultimately a quantitative assessment of TBI enabled by this method will greatly enhance the ability to detect injury suffered, for example, by military personnel in an explosion, monitor progression objectively in a longitudinal study and to predict the clinical outcome of individual TBI patients.

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9. Singh M and Jeong J-W, "**ICA based multi-fiber tractography**" *Proceedings, 17-th Scientific Meeting, Int. Soc. Magnetic Resonance In Medicine,* Hawaii, April 2009a.
10. Singh M and Jeong J-W, **Localization and quantification of injured regions and affected pathways in the 3D head-space of individual TBI subjects using DTI tractography with automatically generated ROIs** *Proceedings, 17-th Scientific Meeting, Int. Soc. Magnetic Resonance In Medicine*, Hawaii, April 2009b.
11. Singh M, Jeong JW, Hwang D, Sungkarat W, Gruen P, **Novel DTI Methodology to Detect and Quantify Injured Regions and Affected Brain Pathways in Traumatic Brain Injury**, *Magnetic Resonance Imaging*, (In review, 2009c).

APPENDIX 1: Localization and quantification of injured regions and affected pathways in the 3D head-space of individual TBI subjects using DTI tractography with automatically generated ROIs

M.Singh and J. Jeong, University of Southern California, Los Angeles, CA

Introduction

Axonal damage is a common occurrence in Traumatic Brain injury (TBI), likely to change local diffusivity and anisotropy measures such as FA in DTI. A *decrease* in FA and an *increase* in mean diffusivity (MD) have been reported by several authors [e.g., [Arfanakis et al. AJNR 23:794,2002] whereas an *increase* in FA and a *decrease* in MD have also been reported [Bazarian et al. J Neurotrauma 24:1447,2007]. Most previous studies rely on a comparison of FA and other anisotropy metrics in hand-drawn Regions of Interest (ROIs) or conduct a voxel based comparison in a normalized space such as the SPM MNI space. Subjectively drawn ROIs are prone to large errors and relating normalized space ROIs to an individual's anatomy is not straightforward. With the emphasis on not requiring any *a priori* hypotheses or manually drawn ROIs, the objectives of this work were to: a) identify and quantify injured regions in a TBI patient in terms of DTI anisotropy metrics changes, and b) identify and quantify the impact of injury on affected brain pathways (tracts). Also as neurosurgical or other interventions rely on the anatomy of the patient's own brain, one of the key goals was to visualize and quantify tractography changes in the patient's own 3D head space.

Method

Whole-brain single shot EPI DTI data were acquired from 12 TBI subjects (traffic accidents, mean age:28 years, mean interval between injury and DTI:1 month) and 10 age-matched normal control human volunteers on a 1.5 T GE EXCITE scanner at TR=10.3s, field-of-view 26cm, 128x128 matrix, 28 contiguous 4mm thick slices using 25 isotropic gradient directions with b=1000s/mm^2, one b=0 acquisition, and number of excitations (NEX)=2 for a total acquisition time of 7min 50s. A customized FA template in MNI space was created by normalizing FA maps of the 10 NC subjects. The center coordinates of MNI space voxels were inverse mapped to each subject's native space to generate an equal number of seeds located at anatomically equivalent locations in each subject for whole-brain tractography (streamline tractography, 0.2mm step size, FA>0.15, deflection<45°). Based on the Jacobian of the transformation matrices, every tract in each control subject was individually transferred first to MNI space and then to the head space of each individual TBI subject. Thus the number of tracts remains unchanged from each control subject to MNI to an individual TBI subject's head. Also this procedure maintains the continuity of individual tracts, does not introduce any additional smoothing, and the inverse seeding strategy normalizes for different head sizes, shapes and individual white-matter variations. An example of normalization is shown in **Fig. 1** where all tracts from 10 controls were transformed to a TBI subject's head and sorted by a common set of filters to extract fronto-occipital tracts. The superposition is excellent in the body of the tracts with expected inter-subject variations as tracts propagate toward cortical areas. To generate ROIs automatically, the FA map of each TBI subject was compared voxel-by-voxel to the FA maps of the control group in MNI space by computing a modified *t*-score defined as: t_i = [FA$_i$ (control) – FA$_i$ (individual)] /σ_i (control) where FA$_i$ (control), FA$_i$ (individual) and σ_i represent mean FA of the control group, FA of an individual and the standard deviation in the FA values of the control group respectively for the *i*-th voxel. This *t*-score distribution in MNI space was then mapped back to the individual subject's head by using inverse normalization, thresholded ($t \geq 3.0$, cluster size $k \geq 12$) and clustered automatically into ROIs relying on contiguity of voxels. In addition to FA, other diffusion anisotropy metrics, e.g., DA, DR, MD were also computed. These ROIs were also used to sort and quantify tracts from the control group and individual TBI subjects where all control group tracts reside in each TBI subject's head. Tract-counts (which reflect connectivity) of a given ROI in each TBI subject were compared to group mean for the same ROI to quantify impact of injury along affected pathways.

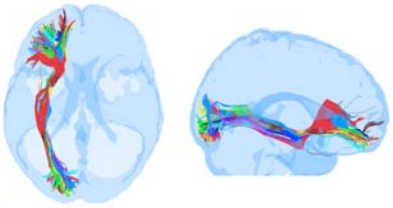


Fig. 1: Normalized fronto-occipital tracts from 10 controls superposed in a TBI subject's head-space.

Results and Discussion

An example of t-score maps corresponding to significant reduction in FA ($t \geq 3.0$, cluster extent $k \geq 12$) between a TBI subject (subject 1) and 10 controls is presented in **Fig. 2**. **Table 1** summarizes the anisotropy metrics and the tract-count reductions in the top 5 ROIs of this subject. Similar results were generated for all TBI subjects and affected pathways were quantified using ROIs as filters. As an example, pathways and corresponding DTI metrics (bar-graph) for the highest *t*-score ROI in TBI subject 1 are shown in **Fig. 3** at FA $\geq$ 0.15. No regions were found where FA would have increased due to injury in this study.

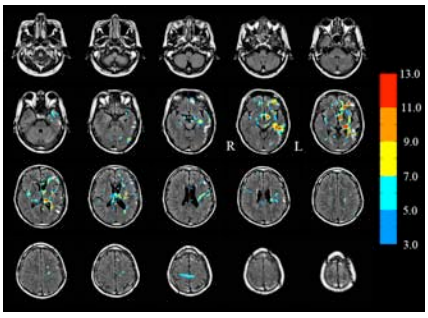
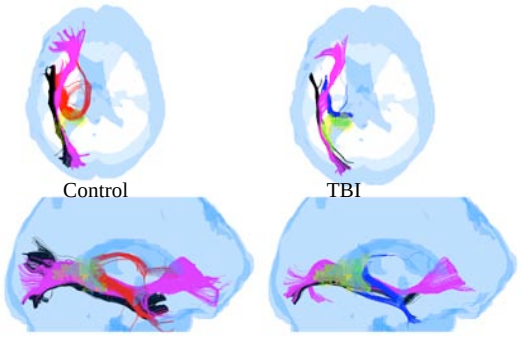


Fig. 2: FA change (t-score in color bar) between TBI subject and 10 controls super-posed on TBI subject's FLAIR images.



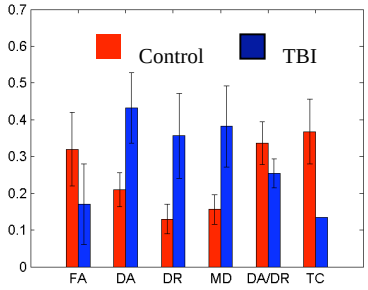


Fig. 3: Three pathways, hippocampal/fornix (HC/FX: red), inferior fronto-occipital (IFO:magenta) and inferior longitudinal fasciculus (ILF: black) in a control and a TBI subject identified by the highest t-score ROI in the TBI subject. DTI metrics incorporating mean and st. dev. of all controls are shown in the graph.

Table1: Summary of DTI and Tract-count (TC) metrics for the top 5 (out of 13) ROIs localized in TBI subject 1 by FA change. t-score is average over ROI.

t-score	Pathway	FA		DA		DR		MD		TC	
		Control	TBI	Control	TBI	Control	TBI	Control	TBI	Control	TBI
	HC/FX-l	0.32(0.10)	0.17(0.11)	1.05(0.23)	2.16(0.48)	0.65(0.20)	1.78(0.58)	0.78(0.20)	1.91(0.55)	367.80(88.58)	135
5.35	IFO-l-1	0.47(0.08)	0.21(0.12)	1.28(0.20)	2.14(0.49)	0.61(0.21)	1.67(0.64)	0.83(0.20)	1.83(0.59)	421.50(114.29)	119
	ILF-l-1	0.49(0.08)	0.24(0.09)	1.18(0.14)	1.84(0.39)	0.53(0.11)	1.35(0.47)	0.74(0.11)	1.51(0.44)	404.30(180.02)	121
5.29	IFO-l-2	0.28(0.06)	0.11(0.04)	0.94(0.07)	1.34(0.18)	0.61(0.07)	1.15(0.18)	0.72(0.06)	1.21(0.18)	409.60(150.97)	62
5.00	CC-g	0.47(0.03)	0.33(0.01)	1.17(0.04)	1.13(0.01)	0.52(0.03)	0.66(0.01)	0.74(0.03)	0.81(0.01)	238.00(55.04)	171
	UF-r	0.42(0.04)	0.31(0.02)	1.12(0.06)	1.10(0.02)	0.56(0.04)	0.67(0.02)	0.75(0.03)	0.81(0.01)	113.80(41.11)	55
4.84	CC-s-2	0.45(0.05)	0.28(0.07)	0.97(0.07)	0.92(0.04)	0.47(0.03)	0.62(0.05)	0.64(0.03)	0.72(0.03)	375.80(116.83)	169
	FX-l	0.29(0.10)	0.16(0.09)	1.24(0.24)	1.85(0.50)	0.83(0.24)	1.54(0.52)	0.97(0.24)	1.64(0.51)	253.50(110.68)	91
4.80	IFO-l-3	0.47(0.07)	0.28(0.07)	1.17(0.11)	1.41(0.23)	0.55(0.07)	0.96(0.26)	0.76(0.07)	1.11(0.24)	347.80(108.94)	74
	ILF-1-2	0.48(0.06)	0.29(0.07)	1.15(0.11)	1.31(0.22)	0.52(0.07)	0.87(0.22)	0.73(0.07)	1.02(0.21)	410.10(181.88)	115

APPENDIX 2: DTI-Tractography to Detect and Quantify Brain Pathways Affected in Traumatic Brain Injury

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Introduction

Accurate diagnosis of traumatic brain injury (TBI) and prediction of outcome following treatment is a key factor in head-trauma management. One of the serious consequences of TBI is diffuse axonal injury (DAI). Though the exact model of axonal damage leading to changes in diffusivity and diffusion anisotropy measures such as fractional anisotropy (FA) is not well-understood, there is converging opinion that DAI represents a progressive injury, beginning with local swelling of axons, followed by cytoskeletal perturbations including misalignment of fibers and eventual disconnection [Arfanakis et al. AJNR 23:794(2002); Bazarian et al. J Neurotrauma 24:1447(2007)]. It has been hypothesized that the consequences of TBI on DTI would be a *decrease* in FA resulting mainly from a decrease in diffusivity along the principal direction. However, an *increase* in FA and a *decrease* in the trace or mean diffusivity have been reported very recently [Bazarian et al. 2007] in a 6-subject study of acute mild TBI. In addition to FA changes, which could be used to locate the injured regions, a thorough evaluation of DAI requires knowledge of specific brain connections that may be disrupted by the injury. The objective of this work was to use DTI to first locate injured regions in individual subjects via changes in the FA compared to a normal group, and then use normalized tractography to quantify disruption along specific brain pathways likely to be affected by the injury.

Method

Whole-brain single shot EPI DTI data were acquired from four TBI subjects (traffic accidents) and 10 age-matched healthy normal control (NC) human volunteers on a 1.5 T GE EXCITE scanner at TR=10.3s, field-of-view 26cm, 128x128 matrix, 28 contiguous 4mm thick slices using 25 isotropic gradient directions with b=1000s/mm^2, one b=0 acquisition, and number of excitations (NEX)=2 for a total acquisition time of 7min 50s. A customized FA template was created by normalizing individual FA maps of the 10 NC subjects through a two-step procedure relying on normalization of segmented white matter voxels (co-registered to the b_0 images) to the MNI white-matter template using a 12 parameter affine/non-linear transformation, followed by refinement in a second step through whole-brain EPI to EPI normalization. FA-template based normalization was then used to map the center points of all voxels in MNI space to each subject's native space by inverse normalization. These inverse mapped coordinates were used as seeds for whole-brain tractography in individual subjects. This procedure ensured that not only were the number of seeds equal in all subjects but also that seeds were distributed at anatomically equivalent locations in the native space of each subject. All tracts from each subject (streamline tractography, 0.2mm step size, FA>0.2, deflection<45°) were then individually transferred back to the MNI space using forward mapping of every 0.2mm spaced point on each tract. Thus the number of tracts remains unchanged from normal to standard space. Also this procedure maintains the continuity of individual tracts, does not introduce any additional smoothing, and normalizes for different head sizes, shapes and individual white-matter variations by distributing an equal number of seeds at anatomical equivalent locations in each subject. Moreover, as tracts from all subjects reside in one common space after normalization, it now becomes possible to isolate actual pathways intersecting any region-of-interest (ROI) in normalized space. Also, as the number of tracts between regions reflects the density of axonal connections between regions, the connectivity between or among specified ROIs for an individual can be quantified by counting tracts between targeted regions. If injured regions contain more voxels whose FA is below the threshold than normals (which is likely in TBI), the number of tracts through the injured regions will also be less than normals. Thus by using the injured regions as ROIs to sort tracts, one can identify specific brain pathways along which connectivity is disrupted by the injury and quantify the loss of connectivity by counting and comparing tracts between individual TBI subjects and normals at a specified FA threshold.

Results and Discussion

An example of a T-score based voxel-based-analysis to detect statistical significant FA changes between a TBI subject and the control group, followed by demarcation of specific brain pathways affected by the top 3 (ranked according to their T-scores) injured regions, is presented in **Fig. 1.**

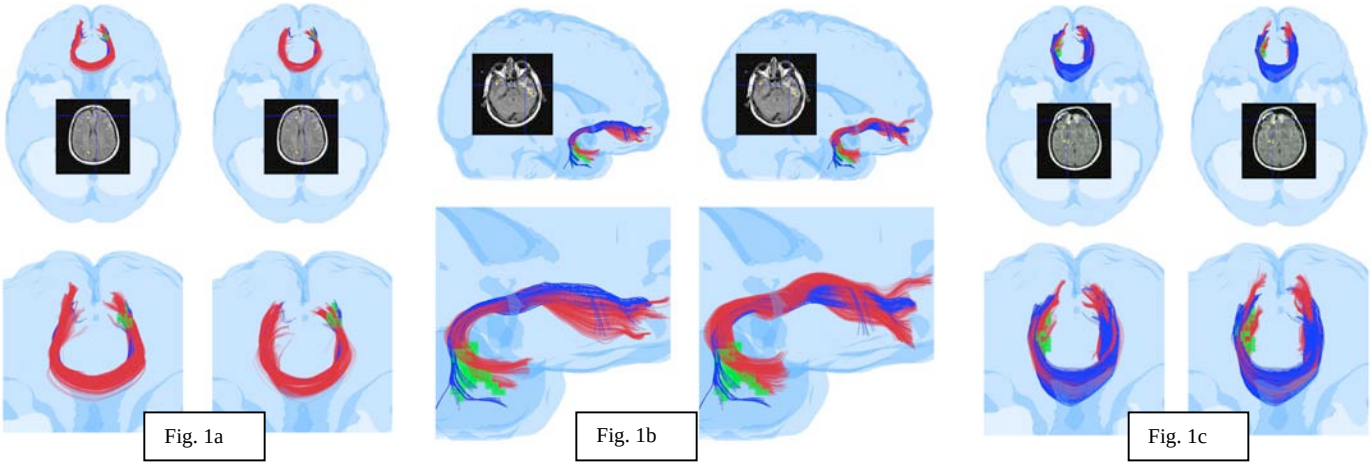

Fig. 1a Fig. 1b Fig. 1c

The inset images show regions (yellow-orange spots) where FA was significantly reduced in a TBI subject ($p<0.001$), superimposed on the subject's FLAIR images. No significant spots were detected where FA would have increased in any of the four TBI subjects. Pathways (FA>0.2) affected by the highest T-score FA-difference region are shown in **Fig. 1a**. The blue tracts are from the TBI subject whereas the red tracts are from two normal subjects (shown at the left and right respectively). The bottom row is a magnified version of the affected pathways. Tracts in the anterior portion of the corpus callosum (genu) are reduced, thus compromising the frontal right-left communications due to this specific injury. **Fig. 1b** similarly shows tracts identified by the second highest FA-difference region (blue for TBI) superposed on the corresponding tracts from two normal subjects (red). This injury is in the temporal lobe (see inset) and the connectivity disruption is along the temporal-frontal pathways. **Fig. 1c** shows sorted tracts from the third highest ROI. This injury is also to the genu and shows further disruption of the right-left connectivity but the specific pathways here are different from those identified by the first ROI.

Results of the tract count along specific pathways are presented in **Fig. 2**. Though there is variability in the connectivity among normal subjects, which is expected, the connectivity along the three specific pathways identified in Fig. 1 is significantly reduced in this example. Similar results were obtained from the other 3 TBI subjects. Interestingly, all had injury to their anterior corpus callosum in addition to several other regions. These results suggest that it is possible to detect and quantify disruptions along specific brain pathways affected by TBI.

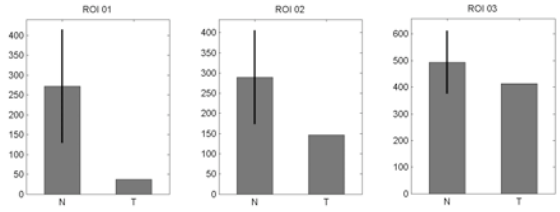

Fig. 2: Comparison of the tract count between normals (N) and the TBI subject (T) along pathways shown in Fig. 1 at FA>0.2. The effect sizes for the connectivity reduction are: 1.66, 1.24 and 0.68 respectively, which are significant large effects.

APPENDIX 3: Detection of White-Matter Pathways affected in TBI by DTI-Tractography

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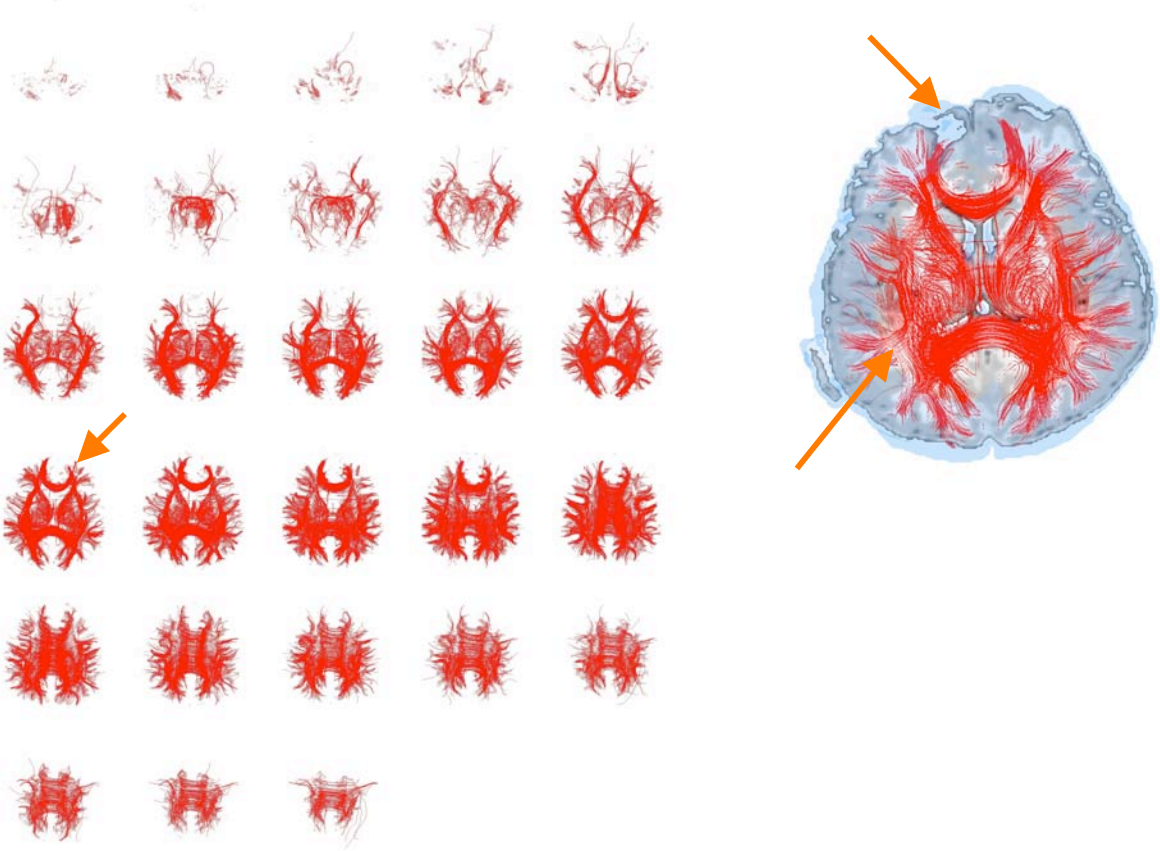
Accurate diagnosis of traumatic brain injury (TBI) and prediction of outcome following treatment is a key factor in head-trauma management. One of the serious consequences of TBI is diffuse axonal injury (DAI) or white matter injury induced by sudden acceleration/deceleration and/or rotational/vibrational forces that cause a shearing of nerve fibers. A thorough evaluation of DAI requires an imaging method sensitive not only to the integrity of axons in white matter but also a knowledge of the network of connections in an individual required to support brain function and also a knowledge of specific networks that may be disrupted by DAI. Using Diffusion Tensor Imaging (DTI) data acquired on a 1.5T MRI scanner from 25 gradient directions for a total data acquisition time of about 7 minutes, we have developed a method to normalize whole-brain white-matter axonal tractography that allows us to perform quantitative comparisons between groups, or between an individual and a group, to detect regions where tracts may be disrupted. A similar method is also used to detect changes in the Fractional Anisotropy (FA) between an individual and a group. Results of a preliminary study with two head trauma patients suggest quantitative detection of changes in FA as well as disruptions in white-matter pathways caused by the traumatic event.



An example of whole-brain tractography for a head-trauma patient. All tracts intersecting a slice are shown for 28 slices. Notice disruptions in tracts of the genu and parietal regions, consistent with the injury in this case. These disruptions are more evident in the rendered version of slice 26 at the right. (The left–right hemispheres are reversed in the rendered version).